THE STRUCTURAL DIFFERENCES IN THE OVA OF *ANOPHELES MACULIPENNIS*, *A. BIFURCATUS* AND *A. PLUMBEUS*

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PLATE XXII

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INTRODUCTION

During investigations which entailed the breeding and rearing of the three species of British Anophelinae at the War Office Laboratory at Sandwich, Kent, in the summer of 1919, I noticed the quite different appearances between the eggs of *Anopheles maculipennis*, *A. bifurcatus*, and *A. plumbeus*, perceptible even to the unaided eye.

There are numerous illustrations of Anopheline ova in the English mosquito literature, but I have seen none indicating the markings on the dorsal surface of the ova of *A. maculipennis* which make it so easy a matter to identify the ova of this species from those of *A. bifurcatus*.

CERTAIN CHARACTERS OF MOSQUITO OVA

The eggs of mosquitoes, although showing much difference in form among the various species, nevertheless have certain characters that are uniform. Generally they are elongated, roughly cigar-shaped, or oval objects. The living elements in the eggs of insects are protected by three separate external coats: (1) a delicate innermost coat termed the vitelline membrane; (2) the hard, shell-like material termed the chorion; and (3) a thin outermost membrane which I shall refer to as the enveloping membrane.

All mosquito ova have the egg-shell proper (chorion) covered or partially covered with the delicate semi-transparent and water-proof enveloping membrane.

This membrane envelopes the egg but is not closely adherent to the shell, a layer of air often existing between the shell and membrane.* Eggs may be easily freed from the membrane by means of pressure with the point of a blunt needle, or by boiling the eggs in water for a moment or two. This membrane may either conform to the surface of the egg and lie so close to the egg-shell as to be only visible when special methods are adopted to demonstrate its presence, or it may, by being 'ballooned', form structures around the egg. Such structures are seen in the 'floats' of Anopheline ova.

The eggs of mosquitoes may be divided into two classes: (1) those that are laid on the water singly, and (2) those that are laid so that the eggs are adherent to each other and float in the form of a raft. In the case of the single eggs these float with their length parallel to the water-surface, and in the case of the raft-eggs they float in a vertical position.

In both classes the individual eggs often have a higher specific gravity than water, and consequently not being particularly buoyant, if submerged and the adherent air detached, they sink to the bottom. The membrane, owing to its water-proof properties, acts as an attachment to the water surface film, causing the egg to float as a dry sewing needle may float when it is placed so gently upon the water that the surface tension of the film is not overcome by the weight of the needle. Naturally, the substance of the egg weighs far less than a proportionate bulk of steel, and the stability of the

* According to Nicholson the membrane has a highly specialised attachment to the chorion.

egg at the surface is thus far greater even in those eggs unprovided with floats.

Nature has apparently recognised that in certain species a greater stability is needed than that afforded to single floating eggs and has developed the plan either of massing the eggs together to form a boat-shaped raft, or by ballooning the enveloping membrane in the single eggs to form the so-called 'floats' so commonly found among the ova of the Anophelinae. The 'floats,' however, do not act to any great extent as true floats giving buoyancy to the eggs, but support them on the surface film securely by presenting a larger area on which the weight of the eggs may be distributed against the surface tension. This fact may be demonstrated by forcibly submerging the eggs, when it will be found that they will either sink or very slowly rise to the surface, generally failing to regain their original position. Mosquito eggs that are laid in raft-formation are almost impossible to submerge without breaking up the raft form and detaching the eggs separately, on account of the air cells formed between the adjacent sides of the eggs. All mosquito eggs are submerged with difficulty, but submersion can be accomplished with the aid of a fine camel's hair brush by simply pressing on the eggs with the brush until each egg is driven below the water surface.

THE OVA OF *ANOPHELES MACULIPENNIS*

The average dimensions of the ova of *A. maculipennis* are, length 0·75 mm., breadth across the widest part of the float 0·27 mm. Eggs from different batches vary in their dimensions slightly, but the average variation is remarkably small. When the eggs are viewed from above while they are floating normally on the water it will be seen that the longitudinal outline of the dorsal surface is roughly cigar-shaped and the floats which lie on each side give the egg a slightly waisted appearance. The dorsal surface is comparatively flattened, while the lower or ventral surface of the egg is evenly rounded. One end of the egg is distinctly blunter and broader than the other. On the ventral side, just below the extremity of the broader end, the micropyle is situated, and it is from the broader end that the larva emerges. The floats are placed on either side of the egg almost at the middle point, though they are actually a little nearer the more pointed end and extend for rather more than a third of the egg's total length.

The float membrane is finely striated transversely, but the striations do not indicate septa dividing the floats into compartments. The membrane which forms the float is superimposed upon the main enveloping membrane, being attached to the main envelope only along the ventro-lateral edges. This separate sheet of membrane which forms the float is ballooned outwards and curved upwards until it touches the dorso-lateral surface of the egg, to which, however, it is not adherent.

By submerging an egg it can be viewed from several aspects. Laid on its side it presents the outline shown in Plate XXII, fig. 1 (*b*), and upside down it has the outline shown in fig. 1 (*c*). It will be noticed that the under surface of the egg is somewhat boat-shaped and draughts progressively less towards the extremities of the egg; also that the enveloping membrane on the under surface of the egg shows hexagonal markings. The enveloping membrane embraces the egg completely except at the micropyle, where a small, round, black area is visible on the under surface of the blunter end of the egg. When the upper surface of the egg of *A. maculipennis* is examined it will be seen that the enveloping membrane spreads out in areas of unequal thickness, presenting, in consequence, dark and light markings as shown in fig. 1 (*a*). In these markings I find a very easy method of distinguishing the eggs of *A. maculipennis* from those of *A. bifurcatus* (compare figs. 1 (*a*) and 2 (*a*)).

The markings are easily seen with a hand lens × 6, and to the unaided eye give the eggs of *A. maculipennis* an ash-grey hue, whereas the eggs of *A. bifurcatus*, having no mottled markings on the dorsal surface, appear almost black in colour. The advantage of being able to distinguish the eggs of these two species from each other is considerable, especially when the species are being bred in the laboratory. Only one cage need be used, in which both species may be kept together; and oviposition may be allowed to take place in the same dish. The ova are then easily separated.

THE OVA OF *ANOPHELES BIFURCATUS*

Average length 0·61 mm.; breadth across the widest part of the float 0·19 mm. In general outline and structure the ova of *A. bifurcatus* closely resemble those of *A. maculipennis*, but the enveloping membrane which cradles the egg to the water line does not extend over the dorsal surface, and the dense black egg-shell is naked. The floats in this species overlap the lateral edges of the egg and give it a distinctly waisted appearance between the floats; moreover, the floats are often of unequal size, and asymmetrical. The two ends of the egg taper to a nearly equal degree, and the curvature of the ventral surface is greater than in the case of the eggs of *A. maculipennis*, causing the ends of the eggs to be more conspicuously upturned.

THE OVA OF *ANOPHELES PLUMBEUS*

The average measurements are, length 0·56 mm.; greatest breadth 0·17 mm. It has been found that to get *A. plumbeus* to tolerate laboratory conditions is far more difficult than with *A. maculipennis* and *A. bifurcatus*. It was only after numerous attempts that I finally succeeded in getting 'wild' specimens to oviposit in the laboratory. These specimens were captured in Epping Forest by exposing ourselves to attack and allowing the mosquitoes to feed. When replete, the mosquitoes were secured by placing a glass tube over them as they rested upon the skin of the arm. They were then transferred to the laboratory and placed in large insect cages containing an almost moisture-saturated atmosphere at a temperature of 30° C. Porcelain photographic developing dishes containing the water from beech tree-holes on which a few dead beech leaves floated were placed in the cages.

Four or five days afterwards the ova of this species were found floating on the water contained in one of the dishes. Owing to the colour of the tree-hole water and the form of the eggs they were exceedingly difficult to see, and when viewed as they floated on the deep brown-coloured water the 'floats' were quite invisible. The eggs were carefully transferred to clean tap-water, and were then seen to be particularly beautiful objects (Plate XXII, fig. 3).

Unlike the ova of *A. maculipennis* and *A. bifurcatus*, the ova of

A. plumbeus are somewhat like the 'diamonds' of playing cards in outline, and are completely surrounded by the floats. The eggs are more pointed at the ends, both ends being alike, and bend slightly upwards. The central part of the upper surface is also raised, and the lower surface is evenly rounded.

It will readily be seen from the illustrations accompanying this paper that the ova of *A. maculipennis*, *A. bifurcatus*, and *A. plumbeus* are so conspicuously different from each other that the differences afford an effective means of identifying the species.

FACTS CONNECTED WITH OVIPOSITION

Anopheles maculipennis.

There is no difficulty in getting the females of this species to oviposit in the laboratory. Specimens captured in pigsties and placed in a large insect cage in the laboratory live well, and will lay several batches of eggs if a guinea-pig is placed in the cage overnight two days after the first and subsequent batches of eggs are laid. The eggs are always laid during the hours of darkness; most often, I think, in the early morning hours before sunrise. I have once obtained a batch of ova at 12 o'clock noon by placing the cage in a dark room.

The eggs when first laid are white, but they usually darken rapidly. Sometimes individual eggs in a batch will, however, remain white and fail to hatch, due, I think, to non-fertilisation. This condition is most readily seen in the ova of females that have been kept in the laboratory for some time and have laid numerous batches of eggs, and it is probably to be explained by a diminished supply of spermatozoa in the spermathecae.

The average batch of eggs laid by *A. maculipennis* in the laboratory has been found to be about one hundred and fifty.

Anopheles bifurcatus.

The eggs of this species are more difficult to obtain, for two reasons. Firstly, the adults are only numerous in many localities between the months of March and the end of May; and secondly, while I have found it easy to get oviposition to take place in the laboratory in the early months of the year, yet specimens captured with difficulty after the end of May and placed under similar conditions cannot be induced to lay, even after they have fed and the ovarian eggs are fully developed. Differences in atmospheric temperature seem to account for this, as I find that as soon as the weather becomes cooler in the autumn and the mosquitoes become more numerous again, the females will readily oviposit. The average batch of eggs from *A. bifurcatus* in the laboratory has been found to be smaller than that of *A. maculipennis*, comprising about one hundred and twenty.

Anopheles plumbeus.

This species has been the most difficult from which to obtain ova. As mentioned previously, repeated attempts had to be made with fed specimens captured in Epping Forest before I was finally successful. So far, I have only managed to obtain three batches at different times from two females. In each case the batch was quite small, consisting of from fifteen to twenty-one ova.

With one of the females the second batch was laid a day later than the first, and this is probably a usual practice with the species to avoid overstocking any one breeding-place, since the majority of tree-holes have only a small water-holding capacity, and comparatively few larvae are to be found in each.

I have recently extended my observations to the ova of the British culicine mosquitoes, and find that in the ova of the species that I have so far examined there are remarkable specific differences sufficiently marked to be seen with the aid of a hand lens × 6, and to enable the identification of the species to be easily made. On this subject a further publication will be made shortly.

Probably owing to the difficulty in getting the majority of the species of mosquitoes to oviposit under laboratory conditions, there is a distinct scarcity of information on the specific differences in the ova. Most of the work on the biology of a species in the existing literature begins with descriptions of the larvae in the first instar, and ends with descriptions of the adult and its life history. The egg stage is, nevertheless, quite as important as any of the life stages—in many ways, from a practical point of view, a particularly important stage. Nuttall and Shipley (1901 and 1903), James and Liston (1911), and Howard, Dyar and Knab (1919) are noteworthy in having given special attention to the description of certain Anopheline ova, but considering how numerous are the species of the *Culicidae*, it may be said that study and description of their ova has been almost neglected.

REFERENCES

GILES, GEO. M. (1902). 'A Handbook of the Gnats or Mosquito.' J. Bale Danielsson & Sons, London.

HOWARD, DYAR and KNAB (1919). 'The Mosquitoes of North America and the West Indies.' Smithsonian Institute, Washington, D.C., U.S.A. Vol. I, p. 140, Vol. II, Plates 145-7.

JAMES, S. P., and LISTON, GLEN W. (1911). 'A Monograph of the Anopheline Mosquitoes of India,' p. 33.

MITCHELL, E. C. (1907). 'Mosquito Life.' Putnam & Sons, New York and London.

NICHOLSON, A. J. (1921). 'On the Development of the Ovary and Ovarian egg of a Mosquito *Anopheles maculipennis*, Meig. *Quarterly Journal of Microscopical Science.* Vol. 65, P. III, pp. 395-448.

NUTTALL and SHIPLEY (1901 and 1903). 'Structure and Biology of Anopheles.' *Journal of Hygiene*, pp. 49-81 and Plate II.

Vol. XV, p. 424, to *List of References* add:

CHRISTOPHERS, S. R. (1916). An Indian tree-hole breeding *Anopheles*, *A. barianensis*, James = *A.* (*coelodiazesis*) *plumbeus*, Haliday. *Ind. Journ. Med. Res.*, Vol. III, p. 489.

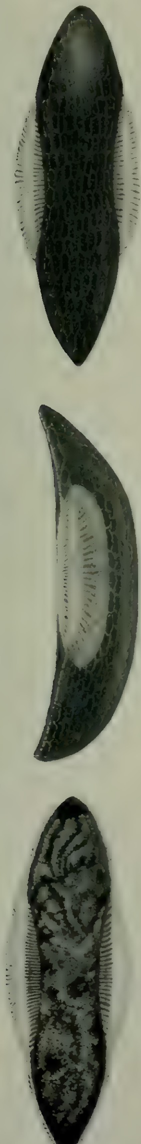
EYSELL, A. (1912). *Cyclophorus* (*Anopheles*) *nigripes*, Staeger, nov. gen. *Archiv. fur Schiffs- und Tropen-Hyg.*, Vol. XVI, p. 421.

EXPLANATION OF PLATE XXII

Fig. 1. Ova of *Anopheles maculipennis:* (*a*) dorsal, (*b*) lateral, (*c*) ventral aspects of ova.

Fig. 2. Ova of *Anopheles bifurcatus:* (*a*) dorsal, (*b*) lateral, (*c*) ventral aspects of ova.

Fig. 3. Ova of *Anopheles plumbeus:* (*a*) dorsal, (*b*) lateral, (*c*) ventral aspects of ova.



c

b

a

FIG. 1

c

b

a

FIG. 2

c

b

a

FIG. 3